

ABSTRACTS

Perilla oil

Suppressing effect of perilla oil on azoxymethane-induced foci of colonic aberrant crypts in rats.

We have investigated the modulatory effect of dietary perilla oil which is rich in the n-3 polyunsaturated fatty acid, alpha-linolenic acid, on the development of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) in male F344 rats. Animals were given three weekly subcutaneous injections of AOM (15 mg/kg body weight) to induce ACE. The rats were fed a basal diet containing either 12% olive oil, 12% safflower oil, 12% perilla oil, 6% perilla oil plus 6% olive oil, or 3% perilla oil plus 9% olive oil for 5 weeks, starting 1 week before the first dosing of AOM. All rats were sacrificed 2 weeks after the last AOM injection. The amount of food consumed and body weight gain were identical among every dietary group. The frequency of ACF was significantly lower in the rats fed 12% perilla oil than in those fed 12% olive oil or 12% safflower oil ($P < 0.01$ and $P < 0.05$, respectively). The suppressive effect of perilla oil was dose-dependent, as the number of ACF was 20.7, 40.7 and 47.4% of those of the 12% olive oil-fed controls in rats fed 12% perilla oil, 6% perilla oil plus 6% olive oil and 3% perilla oil plus 9% olive oil, respectively. Perilla oil significantly reduced ras expression as well as the AgNORs count (cell proliferation biomarkers) in the colonic mucosa, as compared with olive oil or safflower oil ($P < 0.01$, respectively). Marked increases in n-3 polyunsaturated fatty acids in membrane phospholipid fractions and decreased PGE2 levels were observed in colonic mucosa of perilla oil-fed rats. These results suggest that perilla oil, even in small amounts, suppresses the development of aberrant crypt foci, and is therefore a possible preventive agent in the early stage of colon carcinogenesis.

Carcinogenesis. 1996 Jun; 17(6): 1291-6

Colon cancer prevention with a small amount of dietary perilla oil high in alpha-linolenic acid in an animal model.

BACKGROUND. Epidemiologic and experimental studies suggest that dietary fish oil and vegetable oil high in omega-3 polyunsaturated fatty acids (PUFAs) suppress the risk of colon cancer. The optimal amount to prevent colon carcinogenesis with perilla oil high in omega-3 PUFA alpha-linolenic acid in a 12% medium-fat diet was investigated in female F344 rats. For comparison, safflower oil high in omega-6 PUFA linoleic acid was used. **METHODS.** Thirty or 25 rats at 7 weeks of age in each group received an intrarectal dose of 2 mg N-methyl-N-nitrosourea 3 times weekly in weeks 1 and 2 and were fed the diets with various levels of perilla oil and safflower oil throughout the experiment. **RESULTS.** The incidence of colon cancer at the termination of the experiment at week 35 was 40%, 48% and 32% in the rats fed the diets with 3% perilla oil plus 9% safflower oil, 6% perilla oil plus 6% safflower oil, and 12% perilla oil plus 0% safflower oil, respectively, whereas it was 67% in the rats fed the control diet with 0% perilla oil plus 12% safflower oil. The amount of diet consumed and the body weight gain were identical in all of the dietary groups. The ratios of omega-3 PUFA to omega-6 PUFA in the serum and the colonic mucosa at week 35 were increased in parallel to the increased intake of perilla oil. **CONCLUSIONS.** The results suggest that a relatively small fraction of perilla oil, 25% of total dietary fat, may provide an appreciable beneficial effect in lowering the risk of colon cancer.

Cancer. 1994 Apr 15; 73(8): 2069-75

Synergistic suppression of azoxymethane-induced foci of colonic aberrant crypts by the combination of beta-carotene and perilla oil in rats.

The modulating effect of the combined dietary feeding of beta-carotene and perilla oil, which is rich in alpha-linolenic acid, on the development of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) was investigated in male F344 rats. Rats received oral administration of beta-carotene (0, 50 or 200 mg/kg body weight/day) and fed a basal diet containing either 12% olive oil, 3% perilla oil plus 9% olive oil, or 12% perilla oil. A dose-dependent suppressive effect of perilla oil was found. The numbers of ACF were 42.0 and 18.4% of those of the 12% olive oil-fed controls in the rats fed 3% perilla oil plus 9% olive oil and 12% perilla oil, respectively. The development of ACF was also reduced significantly by the addition of dietary beta-carotene in each of the oil-fed groups ($P < 0.05$, respectively). The suppression by the combination of beta-carotene and perilla oil was synergistic, as the numbers of ACF were 12.9 and 8.9% of those of the 12% olive oil-fed controls in beta-carotene-treated rats fed 3% perilla oil plus 9% olive oil and 12% perilla oil, respectively. beta-carotene plus perilla oil also suppressed the numbers of silver-stained nucleolar organizer regions and the expression of ras mRNA in the colonic mucosa (cell proliferation biomarkers). Following administration of beta-carotene, a significant increase in the concentration of intact beta-carotene molecules was found in the colonic mucosa, livers, and sera. However, no accumulation of retinoids was observed in the colonic mucosa, suggesting that the inhibitory effect may not be related to the provitamin A activity. These results suggest that the combination of beta-carotene and perilla oil may be useful in the prevention of colon cancer.

Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial.

This evaluation of the Multiple Risk Factor Intervention Trial database investigated the effects of dietary PUFA on disease outcomes that may relate to polyunsaturated fatty acid (PUFA) biochemistry. The Multiple Risk Factor Intervention Trial was a randomized clinical trial in coronary heart disease (CHD) primary prevention involving 12,866 middle-aged men determined to be at high risk of CHD. They were assigned to either a special intervention group or a usual care group and returned to clinics on an annual basis for assessment of risk factor status. Only data on the usual care men (n = 6,250) are presented, since the multi-intervention effects on the special intervention group introduce considerable analytic complexities. Mean PUFA intake estimates were calculated from four dietary recall interviews at baseline and follow-up Years 1, 2, and 3 and estimates for PUFA were established using absolute grams, percentage of total kilocalories, and ratios. Proportional hazards regression analysis controlling for age, race and baseline diastolic blood pressure, smoking, high and low density lipoprotein cholesterol levels, and alcohol was used to analyze dietary PUFA intakes on 10.5-year mortality rates. Results were more significant when PUFA were expressed as percentage of total kilocalories. No significant associations with mortality were detected for linoleic acid (18:2n-6), the predominant dietary PUFA.

Proc Soc Exp Biol Med. 1992 Jun; 200(2): 177-82

Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States.

OBJECTIVE--To examine the association between fat intake and the incidence of coronary heart disease in men of middle age and older. **DESIGN**--Cohort questionnaire study of men followed up for six years from 1986. **SETTING**--The health professionals follow up study in the United States. **SUBJECTS**--43 757 health professionals aged 40 to 75 years free of diagnosed cardiovascular disease or diabetes in 1986. **MAIN OUTCOME MEASURE**--Incidence of acute myocardial infarction or coronary death. **RESULTS**--During follow up 734 coronary events were documented, including 505 non-fatal myocardial infarctions and 229 deaths. After age and several coronary risk factors were controlled for significant positive associations were observed between intake of saturated fat and risk of coronary disease. For men in the top versus the lowest fifth of saturated fat intake (median = 14.8% v 5.7% of energy) the multivariate relative risk for myocardial infarction was 1.22 (95% confidence interval 0.96 to 1.56) and for fatal coronary heart disease was 2.21 (1.38 to 3.54). After adjustment for intake of fibre the risks were 0.96 (0.73 to 1.27) and 1.72 (1.01 to 2.90), respectively. Positive associations between intake of cholesterol and risk of coronary heart disease were similarly attenuated after adjustment for fibre intake. Intake of linolenic acid was inversely associated with risk of myocardial infarction; this association became significant only after adjustment for non-dietary risk factors and was strengthened after adjustment for total fat intake (relative risk 0.41 for a 1% increase in energy, P for trend < 0.01). **CONCLUSIONS**--These data do not support the strong association between intake of saturated fat and risk of coronary heart disease suggested by international comparisons. They are compatible, however, with the hypotheses that saturated fat and cholesterol intakes affect the risk of coronary heart disease as predicted by their effects on blood cholesterol concentration. They also support a specific preventive effect of linolenic acid intake.

BMJ. 1996 Jul 13; 313(7049): 84-90

Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women.

BACKGROUND: Experimental studies in laboratory animals and humans suggest that alpha-linolenic acid (18:3n-3) may reduce the risk of arrhythmia. **OBJECTIVE:** The objective was to examine the association between dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease (IHD). **DESIGN:** This was a prospective cohort study. The intake of alpha-linolenic acid was derived from a 116-item food-frequency questionnaire completed in 1984 by 76283 women without previously diagnosed cancer or cardiovascular disease. **RESULTS:** During 10 y of follow-up, we documented 232 cases of fatal IHD and 597 cases of nonfatal myocardial infarction. After adjustment for age, standard coronary risk factors, and dietary intake of linoleic acid and other nutrients, a higher intake of alpha-linolenic acid was associated with a lower relative risk (RR) of fatal IHD; the RRs from the lowest to highest quintiles were 1.0, 0.99, 0.90, 0.67, and 0.55 (95% CI: 0.32, 0.94; P for trend = 0.01). For nonfatal myocardial infarction there was only a modest, nonsignificant trend toward a reduced risk when extreme quintiles were compared (RR: 0.85; 95% CI: 0.61, 1.19; P for trend = 0.50). A higher intake of oil and vinegar salad dressing, an important source of alpha-linolenic acid, was associated with reduced risk of fatal IHD when women who consumed this food > or =5-6 times/wk were compared with those who rarely consumed this food (RR: 0.46; 95% CI: 0.27, 0.76; P for trend = 0.001). **CONCLUSIONS:** This study supports the hypothesis that a higher intake of alpha-linolenic acid is protective against fatal IHD. Higher consumption of foods such as oil-based salad dressing that provide polyunsaturated fats, including alpha-linolenic acid, may reduce the risk of fatal IHD.

Am J Clin Nutr. 1999 May; 69(5): 890-7

Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease.

In a prospective, randomised single-blinded secondary prevention trial we compared the effect of a Mediterranean alpha-linolenic acid-rich diet to the usual post-infarct prudent diet. After a first myocardial infarction, patients were randomly assigned to the experimental (n = 302) or control group (n = 303). Patients were seen again 8 weeks after randomisation, and each year for 5

years. The experimental group consumed significantly less lipids, saturated fat, cholesterol, and linoleic acid but more oleic and alpha-linolenic acids confirmed by measurements in plasma. Serum lipids, blood pressure, and body mass index remained similar in the 2 groups. In the experimental group, plasma levels of albumin, vitamin E, and vitamin C were increased, and granulocyte count decreased. After a mean follow up of 27 months, there were 16 cardiac deaths in the control and 3 in the experimental group; 17 non-fatal myocardial infarction in the control and 5 in the experimental groups: a risk ratio for these two main endpoints combined of 0.27 (95% CI 0.12-0.59, $p = 0.001$) after adjustment for prognostic variables. Overall mortality was 20 in the control, 8 in the experimental group, an adjusted risk ratio of 0.30 (95% CI 0.11-0.82, $p = 0.02$). An alpha-linolenic acid-rich Mediterranean diet seems to be more efficient than presently used diets in the secondary prevention of coronary events and death.

Lancet. 1994 Jun 11; 343(8911): 1454-9

Replacement of linoleic acid with alpha-linolenic acid does not alter blood lipids in normolipidaemic men.

The effect of partial dietary replacement of linoleic acid (18:2n-6; linoleic acid-rich diet) with alpha-linolenic acid (18:3n-3; alpha-linolenic acid-rich diet) on plasma lipids was investigated in twenty-nine healthy young men. After a 2-week stabilization period subjects were randomly assigned to either the alpha-linolenic acid-rich diet group (n 15), receiving a mean of 10.1 g of alpha-linolenic acid and 12.1 g of linoleic acid/d, or the linoleic acid-rich diet group (n 14), receiving a mean of 1.0 g of alpha-linolenic acid and 21.0 g of linoleic acid/d, for a 6-week test period. Blood samples were taken at the commencement of the stabilization period and at the start (week 0), midpoint (week 3) and endpoint (week 6) of the test period and plasma lipids analysed. The changes occurring on the linoleic acid-rich diet and alpha-linolenic acid-rich diet were compared but no significant differences in the changes in plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, the subfractions HDL2 and HDL3 or triacylglycerols were found. These results indicate that dietary replacement of linoleic acid with alpha-linolenic acid in the diet of healthy male subjects offers similar cardioprotective benefits with respect to lipid metabolism.

Br J Nutr. 1998 Aug; 80(2): 163-7

Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids.

In animal feeding studies, and probably in humans, n-3 polyunsaturated fatty acids (PUFAs) prevent fatal ischemia-induced cardiac arrhythmias. We showed that n-3 PUFAs also prevented such arrhythmias in surgically prepared, conscious, exercising dogs. The mechanism of the antiarrhythmic action of n-3 PUFAs has been studied in spontaneously contracting cultured cardiac myocytes of neonatal rats. Adding arrhythmogenic toxins (eg, ouabain, high Ca^{2+}), lysophosphatidylcholine, beta-adrenergic agonist, acylcarnitine, and the Ca^{2+} ionophore) to the myocyte perfusate caused tachycardia, contracture, and fibrillation of the cultured myocytes. Adding eicosapentaenoic acid (EPA: 5-15 micromol/L) to the superfusate before adding the toxins prevented the expected tachyarrhythmias. If the arrhythmias were first induced, adding the EPA to the superfusate terminated the arrhythmias. This antiarrhythmic action occurred with dietary n-3 and n-6 PUFAs; saturated fatty acids and the monounsaturated oleic acid induced no such action. Arachidonic acid (AA; 20:4n-6) is anomalous because in one-third of the tests it provoked severe arrhythmias, which were found to result from cyclooxygenase metabolites of AA. When cyclooxygenase inhibitors were added with the AA, the antiarrhythmic effect was like those of EPA and DHA. The action of the n-3 and n-6 PUFAs is to stabilize electrically every myocyte in the heart by increasing the electrical stimulus required to elicit an action potential by approximately 50% and prolonging the relative refractory time by approximately 150%. These electrophysiologic effects result from an action of the free PUFAs to modulate sodium and calcium currents in the myocytes. The PUFAs also modulate sodium and calcium channels and have anticonvulsant activity in brain cells.

Am J Clin Nutr. 2000 Jan; 71(1 Suppl): 202S-7S

Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs.

BACKGROUND: Rat diets high in fish oil have been shown to be protective against ischemia-induced fatal ventricular arrhythmias. Increasing evidence suggests that this may also apply to humans. To confirm the evidence in animals, we tested a concentrate of the free fish-oil fatty acids and found them to be antiarrhythmic. In this study, we tested the pure free fatty acids of the 2 major dietary omega-3 polyunsaturated fatty acids in fish oil: cis-5,8,11,14, 17-eicosapentaenoic acid (C20:5omega-3) and cis-4,7,10,13,16, 19-docosahexaenoic acid (C22:6omega-3), and the parent omega-3 fatty acid in some vegetable oils, cis-9,12,15-alpha-linolenic acid (C18:3omega-3), administered intravenously on albumin or a phospholipid emulsion. **METHODS AND RESULTS:** The tests were performed in a dog model of cardiac sudden death. Dogs were prepared with a large anterior wall myocardial infarction produced surgically and an inflatable cuff placed around the left circumflex coronary artery. With the dogs running on a treadmill 1 month after the surgery, occlusion of the left circumflex artery regularly produced ventricular fibrillation in the control tests done 1 week before and after the test, with the omega-3 fatty acids administered intravenously as their pure free fatty acid. With infusion of the eicosapentaenoic acid, 5 of 7 dogs were protected from fatal ventricular arrhythmias ($P < 0.02$). With docosahexaenoic acid, 6 of 8 dogs were protected, and with alpha-linolenic acid, 6 of 8 dogs were also protected ($P < 0.004$ for each). The before and after control studies performed on the same animal all resulted in fatal ventricular arrhythmias, from which they were defibrillated. **CONCLUSIONS:** These results indicate that purified omega-3 fatty acids can prevent ischemia-induced ventricular fibrillation in this dog model of sudden cardiac death.

Arterial compliance in obese subjects is improved with dietary plant n-3 fatty acid from flaxseed oil despite increased LDL oxidizability.

The compliance or elasticity of the arterial system, an important index of circulatory function, diminishes with increasing cardiovascular risk. Conversely, systemic arterial compliance improves through eating of fish and fish oil. We therefore tested the value of high intake of alpha-linolenic acid, the plant precursor of fish fatty acids. Fifteen obese people with markers for insulin resistance ate in turn four diets of 4 weeks each; saturated/high fat (SHF), alpha-linolenic acid/low fat (ALF), oleic/low fat (OLF), and SHF. Daily intake of alpha-linolenic acid was 20 g from margarine products based on flax oil. Systemic arterial compliance was calculated from aortic flow velocity and aortic root driving pressure. Plasma lipids, glucose tolerance, and in vitro LDL oxidizability were also measured. Systemic arterial compliance during the first and last SHF periods was 0.42 +/- 0.12 (mean +/- SD) and 0.56 +/- 0.21 units based on milliliters per millimeter of mercury. It rose significantly to 0.78 +/- 0.28 (P < .0001) with ALF; systemic arterial compliance with OLF was 0.62 +/- 0.19, lower than with ALF (P < .05). Mean arterial pressures and results of oral glucose tolerance tests were similar during ALF, OLF, and second SHF; total cholesterol levels were also not significantly different. However, insulin sensitivity and HDL cholesterol diminished and LDL oxidizability increased with ALF. The marked rise in arterial compliance at least with alpha-linolenic acid reflected rapid functional improvement in the systemic arterial circulation despite a rise in LDL oxidizability. Dietary n-3 fatty acids in flax oil thus confer a novel approach to improving arterial function.

Arterioscler Thromb Vasc Biol . 1997 Jun; 17(6): 1163-70

Alpha-linolenic acid and cardiovascular diseases.

The intake of saturated fat was postulated to be the main environmental factor for coronary heart disease. It was also postulated that the noxious effects of saturated fatty acids (FA) was primarily through the increase in serum cholesterol. Nevertheless intervention trials either in coronary patients or even in primary prevention did not observe significant reduction in cardiac mortality, especially sudden death, when the diet was markedly enriched in linoleic acid (LA), the most efficient FA to lower serum cholesterol. In intervention trials, it is only when the diet was enriched in n-3 FA, especially alpha-linolenic acid (ALA) that cardiac death was reduced. Studies in animals as well as in vitro on myocytes in culture, have shown that ALA was preventing ventricular fibrillation, the chief mechanism of cardiac death. Furthermore, studies in rats have observed that among n-3 FA, ALA, the precursor of the n-3 family, may be more efficient to prevent ventricular fibrillation than eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition it was demonstrated that ALA was the main FA lowering platelet aggregation, an important step in thrombosis, i. e. non fatal myocardial infarction and stroke. Thus, without side effects, a higher intake of ALA (2g / day) with a ratio of 5/1 for LA/ALA, could possibly constitute a nutritional answer to the main cause of morbidity and mortality in industrialized countries.

J Nutr Health Aging . 2001; 5(3): 179-83

Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation.

We examined the effect of dietary oils with different fatty acid compositions on the growth of visceral adipose tissue in rats. Rats were fed for 4 mo starting at weaning a basal diet containing (12 g/100 g diet) perilla oil rich in (n-3) polyunsaturated fatty acids (PUFA), safflower oil rich in (n-6) PUFA, olive oil rich in monounsaturated fatty acid, or beef tallow rich in saturated fatty acids. The amount of food consumed and body weight gain did not differ among the four dietary groups. The weight of the epididymal fat pad and the serum triglyceride concentration in perilla oil-fed rats were significantly lower (P < 0.05) than those of olive oil- and beef tallow-fed groups. The product of [(volume of individual adipocytes) x (number of adipocytes in epididymal fat pad)], which presumably represents total adipocyte volume in the fat pad, was significantly lower (P < 0.05) in perilla oil-fed rats than in beef tallow- and olive oil-fed groups. Expression of the late genes of adipocyte differentiation, peroxisome proliferator-activated receptor alpha, adipocyte P2 and adipsin, was significantly (P < 0.05) down-regulated in epididymal fat tissue of rats that had been fed perilla oil rather than beef tallow or olive oil, whereas expression of the early gene, lipoprotein lipase, was not significantly affected. Greater levels (P < 0.05) of (n-3) PUFA in the membrane phospholipid fraction of the fat tissue were observed in perilla oil-fed rats than in the other dietary groups. These results suggest that perilla oil or (n-3) PUFA prevents excessive growth of adipose tissue in rats at least in part by suppressing the late phase of adipocyte differentiation.

J Nutr . 1997 Sep; 127(9): 1752-7

N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France.

Experimental studies have indicated that n-3 fatty acids, including alpha-linolenic acid (18:3 n-3) and long-chain n-3 polyunsaturated fatty acids inhibit mammary tumor growth and metastasis. Earlier epidemiological studies have given inconclusive results about a potential protective effect of dietary n-3 polyunsaturated fatty acids on breast cancer risk, possibly because of methodological issues inherent to nutritional epidemiology. To evaluate the hypothesis that n-3 fatty acids protect against breast cancer, we examined the fatty acid composition in adipose tissue from 241 patients with invasive, nonmetastatic breast carcinoma and from 88 patients with benign breast disease, in a case-control study in Tours, central France. Fatty acid

composition in breast adipose tissue was used as a qualitative biomarker of past dietary intake of fatty acids. Biopsies of adipose tissue were obtained at the time of surgery. Individual fatty acids were measured as a percentage of total fatty acids, using capillary gas chromatography. Unconditional logistic regression modeling was used to obtain odds ratio estimates while adjusting for age, height, menopausal status and body mass index. We found inverse associations between breast cancer-risk and n-3 fatty acid levels in breast adipose tissue. Women in the highest tertile of alpha-linolenic acid (18:3 n-3) had an odds ratio of 0.39 (95% confidence intervals [CI] = 0.19-0.78) compared to women in the lowest tertile (trend $p = 0.01$). In a similar way, women in the highest tertile of docosahexaenoic acid (22:6 n-3) had an odds ratio of 0.31 (95% CI = 0.13-0.75) compared to women in the lowest tertile (trend $p = 0.016$). Women in the highest tertile of the long-chain n-3/total n-6 ratio had an odds ratio of 0.33 (95% confidence interval = 0.17-0.66) compared to women in the lowest tertile (trend $p = 0.0002$). In conclusion, our data based on fatty acids levels in breast adipose tissue suggest a protective effect of n-3 fatty acids on breast cancer risk and support the hypothesis that the balance between n-3 and n-6 fatty acids plays a role in breast cancer.

Int J Cancer . 2002 Mar 1; 98(1): 78-83

ABSTRACTS

Resveratrol

Cancer chemopreventive activity of resveratrol, a natural product derived from grapes.

Resveratrol, a phytoalexin found in grapes and other food products, was purified and shown to have cancer chemopreventive activity in assays representing three major stages of carcinogenesis. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes (anti-initiation activity); it mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions (antipromotion activity); and it induced human promyelocytic leukemia cell differentiation (antiprogession activity). In addition, it inhibited the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin cancer model. These data suggest that resveratrol, a common constituent of the human diet, merits investigation as a potential cancer chemopreventive agent in humans.

Science . 1997 Jan 10;275(5297):218-20

Resveratrol: a candidate nutritional substance for prostate cancer prevention.

The dietary stilbene resveratrol is a major constituent of a variety of edible plant products, including grapes and peanuts. Resveratrol has been identified as an excellent candidate cancer chemopreventive, based on its safety and efficacy in animal models of carcinogenesis. Resveratrol is a prototype of a plethora of bioactive polyphenols in the food supply that has just begun to be mined for cancer preventive agents. For example, polyphenolic grape seed fractions were shown recently to potently antagonize chemical carcinogenesis. Taking into consideration that the identification of resveratrol as a cancer preventive agent is largely owed to its high abundance in nature (e.g., it accounts for 5-10% of the grapeskin biomass), it is logical to expect that naturally occurring stilbenes that are superior to resveratrol in their cancer preventive properties await identification. Thus, resveratrol may represent the tip of the iceberg of a broad class of stilbene and related polyphenolic natural products that include safe and highly effective agents for cancer prevention. We hypothesize that resveratrol may be especially suitable as a lead agent for prostate cancer prevention given its ability to: 1) inhibit each stage of multistage carcinogenesis, 2) scavenge incipient populations of androgen-dependent prostate cancer cells through androgen receptor antagonism, and 3) scavenge incipient populations of androgen-independent prostate cancer cells by short-circuiting the epidermal growth factor-receptor (EGFR)-dependent autocrine loops in the cancer cells.

J Nutr . 2003 Jul;133(7 Suppl):2440S-2443S

Inhibition of NF-kappaB pathway in grape seed extract-induced apoptotic death of human prostate carcinoma DU145 cells.

The alarmingly high rate of prostate cancer (PCA) mortality as well as the limited success in the treatment of advanced PCA suggest that additional approaches are needed to control PCA growth and its metastatic potential. A constitutive activation of NF-kappaB family of transcription factors is known to play a major role in chemotherapy resistance in advanced PCA. In recent studies we showed that grape seed extract (GSE) inhibits advanced human PCA growth and induces apoptosis in cell culture and in nude mice. Accordingly, here we assessed the effect of GSE on constitutive and TNFalpha-induced NF-kappaB DNA binding activity and apoptotic death in advanced human prostate carcinoma DU145 cells. Constitutive and TNFalpha-induced NF-kappaB DNA binding activity was inhibited by GSE at doses $>$ or $=$ 50 microg/ml and treatments for $>$ or $=$ 12 h. This was accompanied by inhibition of IkappaBalpha phosphorylation and IKKalpha kinase activity. A strong induction of apoptosis ($P < 0.01$) was also observed following GSE treatment, while a combination with TNFalpha strongly potentiated apoptosis induction. Our results indicate the potential of developing GSE as an effective cancer therapeutic agent, both alone and in combination with TNFalpha-based chemotherapy of advanced human prostate carcinoma that might prove to be a more effective and less toxic alternative in clinical therapy of PCA.

Int J Oncol . 2003 Sep;23(3):721-7

Cancer chemoprevention by resveratrol: in vitro and in vivo studies and the underlying mechanisms (review).

Cancer, next only to heart diseases, is the second leading cause of deaths in the United States of America and many other nations in the world. The prognosis for a patient with metastatic carcinoma of the lung, colon, breast, or prostate (four of the most common and lethal forms of cancer, which together account for more than half of all deaths from cancer in the USA), remains dismal. Conventional therapeutic and surgical approaches have not been able to control the incidence of most of the cancer types. Therefore, there is an urgent need to develop mechanism-based approaches for the management of cancer. Chemoprevention via non-toxic agents could be one such approach. Many naturally occurring agents have shown cancer chemopreventive potential in a variety of bioassay systems and animal models, having relevance to human disease. It is appreciated that an effective and acceptable chemopreventive agent should have certain properties: (a), little or no toxic effects in

normal and healthy cells; (b), high efficacy against multiple sites; (c), capability of oral consumption; (d), known mechanism of action; (e), low cost; and (f), acceptance by human population. Resveratrol is one such agent. A naturally occurring polyphenolic antioxidant compound present in grapes, berries, peanuts and red wine. In some bioassay systems resveratrol has been shown to afford protection against several cancer types. The mechanisms of resveratrol's broad cancer chemopreventive effects are not completely understood. In this review, we present the cancer chemopreventive effects of resveratrol in an organ-specific manner. The mechanisms of the antiproliferative/cancer chemopreventive effects of resveratrol are also presented. We believe that continued efforts are needed, especially well-designed pre-clinical studies in the animal models that closely mimic/represent human disease, to establish the usefulness of resveratrol as cancer chemopreventive agent. This should be followed by human clinical trials in appropriate cancer types in suitable populations.

Int J Oncol . 2003 Jul;23(1):17-28

Glucuronidation of resveratrol, a natural product present in grape and wine, in the human liver.

1. Resveratrol, a polyphenolic compound present in grape and wine, has beneficial effects against cancer and protective effects on the cardiovascular system. It has been shown that the compound is sulphated in human liver and the aims of the present investigation were to study resveratrol glucuronidation in human liver microsomes and to determine whether flavonoids inhibit resveratrol glucuronidation. 2. A simple and reproducible radiometric assay for resveratrol glucuronidation was developed. The assay employed uridine-5'-diphosphoglucuronic acid-[¹⁴C] and unlabelled resveratrol. Resveratrol-glucuronide was isolated by TLC. The intra- and interassays variabilities were 1 and 1.5%, respectively. 3. The rate of resveratrol glucuronidation was measured in 10 liver samples. The mean +/- SD and median of resveratrol glucuronidation rate were 0.69 +/- 0.34 and 0.80 nmol/min/mg, respectively. Resveratrol glucuronosyl transferase followed Michaelis-Menten kinetics and the Km and Vmax (mean +/- SD; n = 5) were 0.15 +/- 0.09 mM and 1.3 +/- 0.3 nmol/min/mg, respectively. The intrinsic clearance was 11 +/- 4 x 10 (-3) ml/min.mg. 4. The flavonoid quercetin inhibited resveratrol glucuronidation and its IC50 (mean +/- SD; n = 3) was 10 +/- 1 microM. Myricetin, catechin, kaempferol, fisetin and apigenin (all at 20 microM) inhibited resveratrol glucuronidation and the percent of control ranged between 46% (catechin) to 72% (apigenin). 5. The present results show that resveratrol is glucuronated in the human liver. Glucuronidation may reduce the bioavailability of this compound however, flavonoids inhibit resveratrol glucuronidation and such an inhibition might improve the bioavailability of resveratrol.

Xenobiotica. 2000 Nov;30(11):1047-54

Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation.

Resveratrol and quercetin are polyphenols which have been detected in significant amounts in green vegetables, citrus fruits and red grape wines. Beneficial effects attributed to these compounds include anti-inflammatory, antiviral and antitumor properties. The effect of resveratrol and quercetin on growth of human oral cancer cells is unknown. Resveratrol and quercetin, in concentrations of 1 to 100 microM, were incubated in triplicates with human oral squamous carcinoma cells SCC-25 in DMEM-HAM's F-12 supplemented with fetal calf serum and antibiotics in an atmosphere of 5% CO₂ in air at 37 degrees C for 72 h. Cell growth was determined by counting the number of viable cells with a hemocytometer. Cell proliferation was measured by means of incorporation of [³H]thymidine in nuclear DNA. Resveratrol at 10 and 100 microM induced significant dose-dependent inhibition in cell growth as well as in DNA synthesis. Quercetin exhibited a biphasic effect, stimulation at 1 and 10 microM, and minimal inhibition at 100 microM in cell growth and DNA synthesis. Combining 50 microM of resveratrol with 10, 25 and 50 microM of quercetin resulted in a gradual and significant increase in the inhibitory effect of quercetin on cell growth and DNA synthesis. We conclude that resveratrol or a combination of resveratrol and quercetin, in concentrations equivalent to that present in red wines, are effective inhibitors of oral squamous carcinoma cell (SCC-25) growth and proliferation, and warrant further investigation as cancer chemopreventive agents.

Anticancer Drugs . 1999 Feb;10(2):187-93

Indole-3-carbinol (I3C) induced cell growth inhibition, G1 cell cycle arrest and apoptosis in prostate cancer cells.

Prostate cancer is one of the most common cancers in men and it is the second leading cause of cancer related death in men in the United States . Recent dietary and epidemiological studies have suggested the benefit of dietary intake of fruits and vegetables in lowering the incidence of prostate cancer. A diet rich in fruits and vegetables provides phytochemicals, particularly indole-3-carbinol (I3C), which may be responsible for the prevention of many types of cancer, including hormone-related cancers such as prostate. Studies to elucidate the role and the molecular mechanism(s) of action of I3C in prostate cancer, however, have not been conducted. In the current study, we investigated whether I3C had any effect against prostate cancer cells and, if so, attempts were made to identify the potential molecular mechanism(s) by which I3C elicits its biological effects on prostate cancer cells. Here we report for the first time that I3C inhibits the growth of PC-3 prostate cancer cells. Induction of G1 cell cycle arrest was also observed in PC-3 cells treated with I3C, which may be due to the observed effects of I3C in the up-regulation of p21(WAF1) and p27(Kip1) CDK inhibitors, followed by their association with cyclin D1 and E and down-regulation of CDK6 protein kinase levels and activity. The induction of p21(WAF1) appears to be transcriptionally upregulated and independent of the p53 responsive element. In addition, I3C inhibited the hyperphosphorylation of the Retinoblastoma (Rb) protein in PC-3 cells. Induction of apoptosis was also observed in this cell line when treated with I3C, as measured by DNA laddering and poly (ADP-ribose) polymersae (PARP) cleavage. We also found an up-regulation of Bax, and down-regulation of Bcl-2 in I3C-treated cells.

These effects may also be mediated by the down-regulation of NF-kappaB observed in I3C treated PC-3 cells. From these results, we conclude that I3C inhibits the growth of PC-3 prostate cancer cells by inducing G1 cell cycle arrest leading to apoptosis, and regulates the expression of apoptosis-related genes. These findings suggest that I3C may be an effective chemopreventive or therapeutic agent against prostate cancer.

Oncogene. 2001 May 24;20(23):2927-36

Grape seed extract inhibits EGF-induced and constitutively active mitogenic signaling but activates JNK in human prostate carcinoma DU145 cells: possible role in antiproliferation and apoptosis.

A loss of functional androgen receptor and an enhanced expression of growth factor receptors and associated ligands are causal genetic events in prostate cancer (PCA) progression. These genetic alterations lead to an epigenetic mechanism where a feedback autocrine loop between membrane receptor and ligand (e.g. EGFR-TGFalpha) results in a constitutive activation of MAPK-Elk1-AP1-mediated mitogenic signaling in human PCA at an advanced and androgen-independent stage. We rationalized that inhibiting these epigenetic events could be useful in controlling advanced PCA growth. Recently, we found that grape seed extract (GSE), a dietary supplement rich in flavonoid procyanidins, inhibits advanced and androgen-independent human PCA DU145 cell growth in culture and nude mice. Here, we performed detailed mechanistic studies to define the effect of GSE on EGFR-Shc-MAPK-Elk1-AP1-mediated mitogenic signaling in DU145 cells. Pretreatment of serum-starved cells with GSE resulted in 70% to almost complete inhibition of EGF-induced EGFR activation and 50% to complete inhibition of Shc activation, which corroborated with a comparable decrease in EGF-induced Shc binding to EGFR. Conversely, EGF-induced ERK1/2 phosphorylation was inhibited only by lower doses of GSE; in fact, higher doses showed an increase. Additional studies showed that GSE alone causes a dose- and time-dependent increase in ERK1/2 phosphorylation in starved DU145 cells that is inhibited by an MEK1 inhibitor PD98059. Independent of this increase in ERK1/2 phosphorylation, GSE showed a strong inhibition of ERK1/2 kinase activity to Elk1 in both cellular and cell-free systems. GSE treatment of cells also inhibited both EGF-induced and constitutively active Elk1 phosphorylation and AP1 activation. GSE treatment also showed DNA synthesis inhibition in starved and EGF-stimulated cells as well as loss of cell viability and apoptotic death that was further increased by adding MEK1 inhibitor. Since GSE strongly induced apoptosis independent of its effect on an increase in phospho-ERK1/2, we hypothesized that apoptotic effect of GSE could be by other mechanism(s) including its effect on stress-associated MAPK, the JNK. Indeed, GSE-treated cells showed a strong and sustained increase in phospho-JNK1/JNK2 levels, JNK activity and phospho-cJun levels. An inhibition of GSE-induced JNK activation by a novel JNK inhibitor SP600125 resulted in a significant reversal of GSE-induced apoptotic death suggesting the involvement of JNK activation by GSE in its apoptosis response. Together, these results suggest that anticancer effects of GSE in PCA be mediated via impairment of EGFR-ERK1/2-Elk1-AP1-mediated mitogenic signaling and activation of JNK causing growth inhibition and apoptosis, respectively.

Oncogene. 2003 Mar 6;22(9):1302-16

Quercetin inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells.

The androgen receptor (AR) is involved in the development and progression of prostate cancer. In order to find new compounds that may present novel mechanisms to attenuate the function of AR, we investigated the effect of a natural flavonoid chemical, quercetin, on androgen action in an androgen-responsive LNCaP prostate cancer cell line. Western blot analysis showed that AR protein expression was inhibited by quercetin in a dose-dependent manner. To demonstrate that the repression effects on AR expression can actually reduce its function, we found that quercetin inhibited the secretion of the prostate-specific, androgen-regulated tumor markers, PSA and hK2. The mRNA levels of androgen-regulated genes such as PSA, NKX3.1 as well as ornithine decarboxylase (ODC) were down-regulated by quercetin. Transient transfections further showed that quercetin inhibited AR-mediated PSA expression at the transcription level. Finally, it was demonstrated that quercetin could repress the expression of the AR gene at the transcription level. Our result suggests that quercetin can attenuate the function of AR by repressing its expression and has the potential to become a chemopreventive and/or chemotherapeutic agent for prostate cancer.

Carcinogenesis . 2001 Mar;22(3):409-1

Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines.

The effect of different wine antioxidant polyphenols (catechin, epicatechin, quercetin, and resveratrol) on the growth of three prostate cancer cell lines (LNCaP, PC3, and DU145) was investigated. A dose- and time-dependent inhibition of cell growth by polyphenols was found at nanomolar concentrations. The proliferation of LNCaP and PC3 cells was preferentially inhibited by flavonoids (catechin, epicatechin, and quercetin), whereas resveratrol was the most potent inhibitor of DU145 cell growth. Possible mechanisms of action were investigated: 1) The competition of polyphenols for androgen binding in LNCaP cells revealed significant interaction only in the case of high concentrations of quercetin, at least at five orders of magnitude higher than the concentrations needed for cell growth inhibition. All other phenols showed low interactions. 2) Oxygen species production after mitogen stimulation and H₂O₂ sensitivity of these cell lines did not correlate with the observed antiproliferative effects, ruling out such a mode of action. 3) NO production revealed two different patterns: LNCaP and DU145 cells produced high concentrations of NO, whereas PC3 cells produced low concentrations. Phorbol ester stimulation of cells did not reveal any additional effect in LNCaP and DU145 cells, whereas it enhanced the secretion of NO in PC3 cells. Polyphenols decreased NO secretion. This effect correlates with their antiproliferative action and the inhibition of inducible NO synthase. It is therefore proposed that the antiproliferative effect of polyphenols is mediated through the modulation of NO production. In conclusion, our

data show a direct inhibitory effect of low concentrations of antioxidant phenols on the proliferation of human prostate cancer cell lines mediated by the production of NO, further suggesting potential beneficial effects of wine and other phenol-containing foods or drinks for the control of prostate cancer cell growth.

Nutr Cancer . 2000;37(2):223-33

Resveratrol pretreatment desensitizes AHTO-7 human osteoblasts to growth stimulation in response to carcinoma cell supernatants.

Resveratrol, a natural phytoestrogen, has been reported to promote differentiation of murine MC3T3-E1 osteoblasts and to inhibit proliferation of prostate cancer cell lines. In the present study we tested the effects of resveratrol on the increased proliferation of human AHTO-7 osteoblastic cell line induced by conditioned media (CM) from a panel of carcinoma cell lines. This compound was found to modulate AHTO-7 proliferation in a tamoxifen-sensitive mechanism at lower concentrations, but failed to induce the osteoblast differentiation marker alkaline phosphatase (ALP) in contrast to vitamin D3. The proliferative response of AHTO-7 cells to conditioned media from carcinoma cell lines was diminished (30-71.4% inhibition) upon pretreatment with 0.5 microM resveratrol. Highest inhibition was demonstrated for pancreas (BxPC3, Panc-1), breast (ZR75-1) and renal (ACHN) carcinoma cell line supernatants whereas the effect on colon carcinoma (SW620, Colo320DM) cell CM and prostate cancer (PC3, DU145 and LNCaP) CM was less pronounced. Direct addition of resveratrol affected only supernatants of cell lines (<25% inhibition) exhibiting growth stimulatory activity for normal WI-38 lung fibroblasts. Resveratrol inhibited proliferation of DU145 and LNCaP cells in concentrations exceeding 5 microM, altered cell cycle distribution of all prostate cancer cell lines in concentrations as low as 0.5 microM, but did not inhibit the production of osteoblastic factors by these lines. In conclusion, resveratrol failed to induce ALP activity as marker of osteoblast differentiation in human osteoblastic AHTO-7 cells, however, inhibited their response to osteoblastic carcinoma-derived growth factors in concentrations significantly lower than those to reduce growth of cancer cells, thus effectively modulating tumor - osteoblast interaction.

Int J Oncol . 1999 Nov;15(5):955-9

Indole-3-carbinol is a negative regulator of estrogen receptor-alpha signaling in human tumor cells.

Estrogen, via its binding to the estrogen receptor (ER), plays an important role in breast cancer cell proliferation and tumor development. Indole-3-carbinol (I3C), a compound occurring naturally in cruciferous vegetables, exhibits a potent antitumor activity via its regulation of estrogen activity and metabolism. This study was designed to determine the effect of I3C on the potential to inhibit the ER-alpha. Using a reporter gene driven by the estrogen receptor, I3C (10-125 micromol/L) significantly repressed the 17ss-estradiol (E2)-activated ER-alpha signaling in a dose-dependent manner. I3C and breast cancer susceptibility gene 1 (BRCA1) synergistically inhibited transcriptional activity of ER-alpha. Moreover, I3C down-regulated the expression of the estrogen-responsive genes, pS2 and cathepsin-D, and up-regulated BRCA1. The inhibitory effects of I3C did not contribute to its cytotoxic effects because these activities were observed at less than toxic concentrations. These results further suggest that antitumor activities of I3C are associated not only with its regulation of estrogen activity and metabolism, but also its modulation of ER transcription activity.

J Nutr . 2000 Dec;130(12):2927-31

Cytostatic and antiestrogenic effects of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane, a major in vivo product of dietary indole-3-carbinol.

Under acidic conditions, indole-3-carbinol (I3C) is converted to a series of oligomeric products thought to be responsible for the biological effects of dietary I3C. Chromatographic separation of the crude acid mixture of I3C, guided by cell proliferation assay in human MCF-7 cells, resulted in the isolation of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane (LTr-1) as a major antiproliferative component. LTr-1 inhibited the growth of both estrogen-dependent (MCF-7) and -independent (MDA-MB-231) breast cancer cells by approximately 60% at a non-lethal concentration of 25 microM. LTr-1 had no apparent effect on the proliferation of MCF-7 cells in the absence of estrogen. LTr-1 was a weak ligand for the estrogen receptor (ER) (IC50 70 microM) and efficiently inhibited the estradiol (E2)-induced binding of the ER to its cognate DNA responsive element. The antagonist effects of LTr-1 also were exhibited in assays of endogenous pS2 gene expression and in cells transiently transfected with an estrogen-responsive reporter construct (pERE-vit-CAT). LTr-1 activated both binding of the aryl hydrocarbon (Ah) receptor to its cognate DNA responsive element and expression of the Ah receptor-responsive gene CYP1A1. LTr-1 was a competitive inhibitor of CYP1A1-dependent ethoxyresorufin-O-deethylase (EROD) activity. In summary, these results demonstrated that LTr-1, a major in vivo product of I3C, could inhibit the proliferation of both estrogen-dependent and -independent breast tumor cells and that LTr-1 is an antagonist of estrogen receptor function and a weak agonist of Ah receptor function.

Biochem Pharmacol . 1999 Sep 1;58(5):825-34

Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells.

3,3'-Diindolylmethane (DIM) is a major digestive product of indole-3-carbinol, a potential anticancer component of cruciferous vegetables. Our results indicate that DIM exhibits potent antiproliferative and antiandrogenic properties in androgen-dependent human prostate cancer cells. DIM suppresses cell proliferation of LNCaP cells and inhibits dihydrotestosterone (DHT) stimulation

of DNA synthesis. These activities were not produced in androgen-independent PC-3 cells. Moreover, DIM inhibited endogenous PSA transcription and reduced intracellular and secreted PSA protein levels induced by DHT in LNCaP cells. Also, DIM inhibited, in a concentration-dependent manner, the DHT-induced expression of a prostate-specific antigen promoter-regulated reporter gene construct in transiently transfected LNCaP cells. Similar effects of DIM were observed in PC-3 cells only when these cells were co-transfected with a wild-type androgen receptor expression plasmid. Using fluorescence imaging with green fluorescent protein androgen receptor and Western blot analysis, we demonstrated that DIM inhibited androgen-induced androgen receptor (AR) translocation into the nucleus. Results of receptor binding assays indicated further that DIM is a strong competitive inhibitor of DHT binding to the AR. Results of structural modeling studies showed that DIM is remarkably similar in conformational geometry and surface charge distribution to an established synthetic AR antagonist, although the atomic compositions of the two substances are quite different. Taken together with our published reports of the estrogen agonist activities of DIM, the present results establish DIM as a unique bifunctional hormone disrupter. To our knowledge, DIM is the first example of a pure androgen receptor antagonist from plants.

J Biol Chem . 2003 Jun 6;278(23):21136-45. Epub 2003 Mar 27.

Distinct forms of hepatic androgen 6 beta-hydroxylase induced in the rat by indole-3-carbinol and pregnenolone carbonitrile.

The ability of indole-3-carbinol (IC), an anticarcinogen present in cruciferous vegetables, to induce CYP1A1, CYP1A2, CYP2B1/2, CYP2E1 and CYP3A1/2 in female rat liver was determined by Western analysis using monoclonal antibodies and compared to effects produced by pregnenolone carbonitrile in animals of both sexes. The ontogeny of induction of these cytochrome P450 isozymes in response to oral administration of IC was also investigated. An inverse correlation was observed between the 6 beta-hydroxylation of androsterone (A) and the induction by IC of CYP3A1/2, the P450 isozyme responsible for the bulk of hepatic 6 beta-hydroxylation of 4-androstenedione (AD). The effect of inhibitors on the formation of 6 beta-OHA from A or AD was also determined and shown to differ from their action on the P450 isozymes involved in the formation of the 6 beta-hydroxylated derivatives of AD or lithocholic acid. The results indicate that the enzyme induced by IC is distinct from the CYP3A1/2 which catalyzes hydroxylations at position 6 beta, allylic in AD but not in the fully saturated ring system of A. The increased hepatic conversion of A to its biologically less active 6 beta-OHA metabolite after treatment of female rats with IC could possibly contribute to the anticarcinogenic action of indole carbinols. It is also proposed that the action of multiple inducers present in cruciferous and other vegetables might produce androgen metabolic profiles very different from those produced by individual components isolated from them.

J Steroid Biochem Mol Biol . 1994 Nov;51(3-4):219-25

Acid reaction products of indole-3-carbinol and their effects on cytochrome P450 and phase II enzymes in rat and monkey hepatocytes.

The effects of three acid condensation products of indole-3-carbinol (I3C), i.e. 3,3'-diindolylmethane (DIM), 5,6,11,12,17,18-hexahydrocyclohepta[1,2-b:4,5-b':7,8-b'']tri-indole (CTI) and 2,3-bis[3-indolylmethyl]indole (BII), on cytochrome P450 and phase II enzymes were studied in primary cultures of rat and cynomolgus monkey liver cells. In rat hepatocytes all three indole derivatives dose-relatedly induced the ethoxyresorufin O-dealkylation (EROD) activity (to 24-fold) and 7 alpha-hydroxylation of testosterone (to 4-fold), whereas all three decreased the 16 alpha- and 2 alpha-testosterone hydroxylation (DIM to 60%, CTI and BII to a mere 5% of the control cells). Treatment of monkey hepatocytes with DIM and BII enhanced the EROD activity to 6- and 9-fold, respectively. Furthermore, BII decreased the 6 beta-hydroxylation of testosterone (to 60% of the untreated cultures) in monkey cells. Phase II enzymes were also affected. In rat hepatocytes DIM, CTI and BII enhanced DT-diaphorase (DTD) (= NAD(P)H-quinone reductase) activity, and DIM and BII the glucuronidation of 1-naphthol. In monkey cells BII only enhanced DTD, and no changes were observed in the glucuronidation of 1-naphthol after treatment with either DIM or BII. The indole derivatives did not affect glutathione S-transferase activity and sulfation of 1-naphthol in either rat or monkey hepatocytes. These results identify two novel acid condensation products of I3C, CTI and BII, as potent compounds in affecting biotransformation in rat as well as in monkey hepatocytes.

Biochem Pharmacol . 1992 Apr 1;43(7):1439-47

Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells.

Resveratrol is a natural phytoalexin compound found in grapes and other food products. In this study, the effect of resveratrol on the growth of human breast cancer cells was examined. Results show that resveratrol inhibits the growth of estrogen receptor (ER)-positive MCF-7 cells in a dose-dependent fashion. Detailed studies with MCF-7 cells demonstrate that resveratrol antagonized the growth-promoting effect of 17-beta-estradiol (E2) in a dose-dependent fashion at both the cellular (cell growth) and the molecular (gene activation) levels. At 5×10^{-6} M, resveratrol abolished the growth-stimulatory effect mediated by concentrations of E2 up to 10^{-9} M. The antiestrogenic effect of resveratrol could be observed at a concentration of 10^{-6} M and above. The antiestrogenic effect of resveratrol was also demonstrated at the molecular level. Resveratrol in a dose-dependent fashion antagonized the stimulation by E2 of progesterone receptor gene expression in MCF-7 cells. Moreover, expression of transforming growth factor-alpha and insulin-like growth factor I receptor mRNA was inhibited while the expression of transforming growth factor beta2 mRNA was significantly elevated in MCF-7 cells cultivated in the presence of resveratrol (10^{-5} M). In

summary, our results show that resveratrol, a partial ER agonist itself, acts as an ER antagonist in the presence of estrogen leading to inhibition of human breast cancer cells.

J Cell Physiol . 1999 Jun;179(3):297-304

ABSTRACTS

Hydroderm

Modulation of skin collagen metabolism in aged and photoaged human skin in vivo.

To the best of our knowledge, no study has been conducted to date to directly compare the collagen metabolism of photoaged and naturally aged human skin. In this study, we compared collagen synthesis, matrix metalloproteinase-1 levels, and gelatinase activity of sun-exposed and sun-protected skin of both young and old subjects. Using northern blot analysis, immunohistochemical stain, and Western blot analysis, we demonstrated that the levels of procollagen type I mRNA and protein in photoaged and naturally aged human skin in vivo are significantly lower than those of young skin. Furthermore, we demonstrated, by northern blot analysis, that the procollagen alpha1(I) mRNA expression of photoaged skin is much greater than that of sun-protected skin in the same individual. In situ hybridization and immunohistochemical stain were used to show that the expression of type I procollagen mRNA and protein in the fibroblasts of photoaged skin is greater than for naturally aged skin. In addition, it was found, by Western blot analysis using protein extracted from the dermal tissues, that the level of procollagen type I protein in photoaged skin is lower than that of naturally aged skin. The level of matrix metalloproteinase-1 protein and the activity of matrix metalloproteinase-2 were higher in the dermis of photoaged skin than in naturally aged skin. Our results suggest that the natural aging process decreases collagen synthesis and increases the expression of matrix metalloproteinases, whereas photoaging results in an increase of collagen synthesis and greater matrix metalloproteinase expression in human skin in vivo. Thus, the balance between collagen synthesis and degradation leading to collagen deficiency is different in photoaged and naturally aged skin.

J Invest Dermatol. 2001 Nov;117(5):1218-24

Mathematical models describing polymer dissolution: consequences for drug delivery.

Polymer dissolution is an important phenomenon in polymer science and engineering that has found applications in areas like microlithography, controlled drug delivery, and plastics recycling. This review focuses on the modeling efforts to understand the physics of the drug release process from dissolving polymers. A brief review of the experimentally observed dissolution behavior is presented, thus motivating the modeling of the mechanism of dissolution. The main modeling contributions have been classified into two broad approaches - phenomenological models and Fickian equations, and anomalous transport models and scaling law-based approaches. The underlying principles and the important features of each approach are discussed. Details of the important models and their corresponding predictions are provided. Experimental results seem to be qualitatively consistent with the present picture.

Adv Drug Deliv Rev. 2001 Jun 11;48(2-3):195-210

Enhanced skin permeation of a lipophilic drug using supersaturated formulations.

Supersaturation was used to enhance the permeation of a lipophilic model compound (a lavendustin derivative, LAP) through excised pig skin in vitro. The drug was dissolved in a series of liquid and semisolid vehicles (in which it had different solubilities) and which were prepared using either (i) the method of mixed cosolvents, (ii) the method of solvent evaporation, or (iii) the method of dissolving the drug with heating. Saturated formulations showed comparable permeation rates through the skin, independent of the absolute concentration of the drug in the vehicle. Supersaturated solutions at a degree of saturation of two resulted in a doubling of the drug permeation rate. These experiments show, therefore, that the percutaneous absorption of LAP may be consistently increased using supersaturated formulations, independent of the type and composition of the vehicles and independent of their method of preparation.

J Control Release. 2001 Jun 15;73(2-3):245-53

Image analysis of dermal collagen changes during skin aging.

OBJECTIVE: To determine progressive quantitative, directional and textural changes in dermal collagen as a function of age and sex and to estimate their evolutive trend with appropriate regression models. **STUDY DESIGN:** Ninety-six samples of abdominal skin from autopsy cases were analyzed. The ages ranged from 3.5 months to 86 years. Picro-Sirius-stained slides were examined by polarizing microscopy, and spatial density, directional features and texture of collagen were measured by computerized image analysis. Nonlinear regression models were built to estimate evolutive changes with respect to age. The relationship between spatial orientation of collagen bundles and age was best modeled by linear regression. **RESULTS:** The evolutive patterns of dermal thickness and spatial density of collagen bundles correspond to a second-order polynomial model with a progressive increase from childhood to middle age and a relatively sharp decrease after the seventh decade. The evolution of textural pattern of dermal collagen, defined by gradient analysis, depicts a sort of inverted U. Its complexity is maximum in the first year of life, decreases until the period 25-50 years and increases progressively after the sixth decade. The horizontal orientation of collagen bundles with intermingled fascicles oriented in other directions, shown by young individuals, is progressively simplified with aging. In elderly subjects, collagen bundles have a horizontal orientation. No significant sex-related

differences were found. CONCLUSION: Dermal collagen changes related to aging are apparently independent of sex, at least in abdominal skin, and show characteristic curvilinear evolutive trends defined by decreased dermal thickness in the elderly, decrease in the spatial density of collagen bundles and increase in textural heterogeneity of the dermis. Progressive simplification in the orientation of collagen bundles leading to a predominant horizontal disposition followed a linear trend. These changes could contribute to providing a substantial morphologic basis to age-associated biomechanical alterations in the skin.

Anal Quant Cytol Histol . 1998 Dec;20(6):493-9

Aging of the extracellular matrix and its pathology.

Recent concepts on the mechanisms of aging of extracellular matrix (EM) are reviewed as well as its involvement in age-associated diseases. Cell differentiation, histogenesis and organogenesis can be analyzed in terms of the program of the biosynthesis of EM macromolecules during development, maturation and aging. The most important biological role of EM is the integration of cells in tissues, of tissues in organs and of organs in the whole organism. EM can directly influence cell behavior through the contact between EM and the genome mediated by structural glycoproteins (fibronectin, laminin, elastin, etc.) interacting with other EM macromolecules (collagen, proteoglycans, elastin) and the cytoskeleton by trans-membrane receptors (integrins). Most age-associated diseases exhibit a deviation (qualitative or quantitative) from the normal program of EM biosynthesis. Three examples are analyzed in some detail: atherosclerosis, diabetes and malignant tumors. The degradation of elastic fibers catalyzed by cellular elastase-type enzymes is observed in atherosclerosis and also in emphysema and skin aging. Several of these enzymes were isolated and characterized from platelets, fibroblasts, smooth muscle cells and lipoproteins. The biosynthesis of some of them increases with age and facilitates cell migration. Plasma fibronectin increases with age exponentially. This increase is absent or strongly attenuated in diabetes and some cancers. Tissue fibronectin increases in diabetes, Werner syndrome and in the peritumoral desmoplastic reaction while most tumor cells can no more retain fibronectin on their membrane facilitating their movement in the organism. These examples demonstrate the importance of the study of cell matrix interactions for gerontology.

Exp Gerontol. 1988;23(1):5-18

Fifty years of skin aging.

In developed countries, interest in cutaneous aging is in large part the result of a progressive, dramatic rise over the past century in the absolute number and the proportion of the population who are elderly (Smith et al, 2001). The psychosocial as well as physiologic effects of skin aging on older individuals have created a demand for better understanding of the process and particularly for effective interventions. Skin aging is a complex process determined by the genetic endowment of the individual as well as by environmental factors. The appearance of old skin and the clinical consequences of skin aging have been well known for centuries, but only in the past 50 y have mechanisms and mediators been systematically pursued. Still, within this relatively short time there has been tremendous progress, a progress greatly enhanced by basic gerontologic research employing immunologic, biochemical, and particularly molecular biologic approaches.

J Investig Dermatol Symp Proc . 2002 Dec;7(1):51-8

Physiological consequences of human skin aging.

The expression and treatment of cutaneous disease in the elderly differ from those applicable to younger adults. Anatomical changes in aging skin result in altered physiological behavior and susceptibility to disease. Decreased epidermal renewal and tissue repair accompany the aging process. The rate of hair and nail growth declines, as well as the quantity of eccrine, apocrine, and sebum secretion. There are alterations in immune surveillance and antigen presentation with aging. The cutaneous vascular supply is decreased, leading to decreases in inflammatory response, absorption, and cutaneous clearance. Impaired thermal regulation, tactile sensitivity, and pain perception occur as one ages. We summarize the major changes that occur during the intrinsic aging process of the skin to facilitate the recognition and treatment of skin disease in the older patient.

Cutis. 1989 May;43(5):431-6

Histologic changes in skin associated with aging.

This is a review of histologic changes noted in the skin of elderly individuals. Among the epidermal changes associated with skin aging are a flattened dermal-epidermal junction, giving the appearance of atrophy and cellular heterogeneity. The melanocyte density declines slowly, and the Langerhans cells decrease in number with advancing age. Among the dermal changes are attenuation in the number and diameter of elastic fibers in the papillary dermis, an increase in number and thickness of the same fibers in the reticular dermis, and a coarsening of collagen fibers with an increase in density of the collagen network. A decrease in the dermal cell population as well as a functional decline in glandular activity are also noted with intrinsic aging. A decline in hair number, rate of growth, and diameter, along with a slowing of the rate of growth of nails, have been well documented with progressive aging.

J Dermatol Surg Oncol . 1990 Oct;16(10):908-14

Aging and cross-linking of skin collagen.

This report represents a clear demonstration of a cross-link in collagen whose abundance is related to chronological aging of an organism. Recently its structure was identified as histidinohydroxylysinonorleucine. Quantification of the cross-link in various aged samples of bovine and human skin indicate that it rapidly increases from birth through maturation. Subsequently, a steady increase occurs with aging, approaching 1 mole/mole of collagen. This compound seems to be related to the relative proportions of soluble to insoluble collagen from skin in neutral salt, dilute acid, and denaturing aqueous solvents (higher concentration in the insoluble portion). It is absent from other major collagenous tissues such as dentin, bone and tendon.

Biochem Biophys Res Commun . 1988 Apr 29;152(2):898-903

Connective tissue biochemistry of the aging dermis. Age-associated alterations in collagen and elastin.

Cutaneous aging represents a complex situation in which at least two independent factors--innate aging and solar exposure--contribute to the development of degenerative changes in the dermis. The biochemical and ultrastructural evidence reviewed in this article indicates that reduced collagen deposition, as a result of diminished collagen biosynthesis and reduced proliferative capacity of the fibroblasts, could explain the development of dermal atrophy and would relate to poor wound healing in the elderly. At the same time, perturbations in the supramolecular organization of the elastic fiber network lead to alterations in the mechanical properties of the skin, as manifested by loose and sagging skin with reduced resilience and elasticity.

Clin Geriatr Med . 1989 Feb;5(1):127-47

Factors of skin ageing share common mechanisms.

Ageing has been defined as the accumulation of molecular modifications which manifest as macroscopic clinical changes. Human skin, unique among mammals insofar as it is deprived of fur, is particularly sensitive to environmental stress. Major environmental factors have been recognized to induce modifications of the morphological and biophysical properties of the skin. Metabolites from ingested or inhaled substances do affect skin, which is also sensitive to endogenous hormone levels. Factors as diverse as ultraviolet radiation, atmospheric pollution, wounds, infections, traumatism, anoxia, cigarette smoke, and hormonal status have a role in increasing the rate of accumulation of molecular modifications and have thus been termed 'factors of ageing'. All these factors share as a common feature, the capability to directly or indirectly induce one of the steps of the micro-inflammatory cycle, which includes the expression of ICAM-1 in endothelial cells. This triggers a process leading to the accumulation of damages in the skin resulting in skin ageing since ICAM-1 expression provokes recruitment and diapedesis of circulating immune cells, which digest the extracellular matrix (ECM) by secreting collagenases, myeloperoxidases and reactive oxygen species. The activation of these lytic processes provokes random damage to resident cells, which in turn secrete prostaglandines and leukotrienes. These signaling molecules induce the degranulation of resident mast cells which release the autacoid histamine and the cytokine TNF-alpha thus activating endothelial cells lining adjacent capillaries which release P-selectin and synthesize ICAM-1. This closes a self-maintained micro-inflammatory cycle, which results in the accumulation of ECM damage, i.e. skin ageing. In this paper we review the evidence that two factors able to induce macroscopical and molecular modifications in the skin, protein glycation and stretch, activate the micro-inflammatory cycle. We further present evidence that three additional factors, two external factors (electromagnetic fields and psychological stressors) and one internal factor (neuropeptides) also activate the micro-inflammatory cycles and may therefore be considered as factors of skin ageing.

Biogerontology. 2001;2(4):219-29

ABSTRACTS

Testosterone

Transdermal testosterone treatment in women with impaired sexual function after oophorectomy.

BACKGROUND: The ovaries provide approximately half the circulating testosterone in premenopausal women. After bilateral oophorectomy, many women report impaired sexual functioning despite estrogen replacement. We evaluated the effects of transdermal testosterone in women who had impaired sexual function after surgically induced menopause. **METHODS:** Seventy-five women, 31 to 56 years old, who had undergone oophorectomy and hysterectomy received conjugated equine estrogens (at least 0.625 mg per day orally) and, in random order, placebo, 150 microg of testosterone, and 300 microg of testosterone per day transdermally for 12 weeks each. Outcome measures included scores on the Brief Index of Sexual Functioning for Women, the Psychological General Well-Being Index, and a sexual-function diary completed over the telephone. **RESULTS:** The mean (+/-SD) serum free testosterone concentration increased from 1.2+/-0.8 pg per milliliter (4.2+/-2.8 pmol per liter) during placebo treatment to 3.9+/-2.4 pg per milliliter (13.5+/-8.3 pmol per liter) and 5.9+/-4.8 pg per milliliter (20.5+/-16.6 pmol per liter) during treatment with 150 and 300 microg of testosterone per day, respectively (normal range, 1.3 to 6.8 pg per milliliter [4.5 to 23.6 pmol per liter]). Despite an appreciable placebo response, the higher testosterone dose resulted in further increases in scores for frequency of sexual activity and pleasure-orgasm in the Brief index of Sexual Functioning for Women (P=0.03 for both comparisons with placebo). At the higher dose the percentages of women who had sexual fantasies, masturbated, or engaged in sexual intercourse at least once a week increased two to three times from base line. The positive-well-being, depressed-mood, and composite scores of the Psychological General Well-Being Index also improved at the higher dose (P=0.04, P=0.03, and P=0.04, respectively, for the comparison with placebo), but the scores on the telephone-based diary did not increase significantly. **CONCLUSIONS:** In women who have undergone oophorectomy and hysterectomy, transdermal testosterone improves sexual function and psychological well-being.

N Engl J Med . 2000 Sep 7;343(10):682-8

Androgen replacement in women: a commentary.

There is increasing evidence to suggest that many postmenopausal women experience symptoms alleviated by androgen therapy and that such symptoms may be secondary to androgen deficiency. Affected women complain of fatigue, low libido, and diminished well-being, symptoms easily and frequently attributed to psychosocial and environmental factors. When such symptoms occur in the setting of low circulating bioavailable testosterone, testosterone replacement results in significant improvement in symptomatology and, hence, quality of life for the majority of women. Whether the apparent therapeutic effects of testosterone replacement are mediated by testosterone and its metabolite 5alpha-dihydrotestosterone or are a consequence of aromatization to estrogen is not known. Despite the paucity of data regarding its effects, inclusion of testosterone in postmenopausal hormone replacement regimens is not uncommon and is likely to become more widespread with the availability of preparations developed specifically for women. Other novel and even more controversial potential indications for androgen therapy in women are currently being evaluated. These include use in women with premature ovarian failure, premenopausal androgen deficiency symptoms, postmenopausal and glucocorticosteroid-related bone loss, alleviation of wasting syndrome secondary to human immunodeficiency virus infection, and management of premenstrual syndrome. The aim of this commentary is to very briefly review the rationale for the use of testosterone in women, create awareness of some of the therapeutic options available in various countries, and stimulate discussion of this important aspect of women's health.

J Clin Endocrinol Metab. 1999 Jun;84(6):1886-91

Androgens and female sexuality.

An accumulating body of data indicates that many women experience a cluster of symptoms that are responsive to testosterone treatment and may be due to androgen deficiency. Characteristically, affected women complain of low libido, persistent fatigue, and diminished well-being and are found to have low circulating bioavailable testosterone. Whether the apparent therapeutic effects of testosterone are mediated via the androgen receptor or as a consequence of metabolism to estrogen is not known. Despite the lack of understanding of the mechanism(s) by which testosterone may enhance libido, the prescription of testosterone to women in a variety of formulations is becoming increasingly popular. This article provides an overview of the rationale for testosterone therapy in women, offers a broad definition of androgen deficiency in women based on the clinical experience of the author, and outlines the currently available options and potential risks of testosterone replacement in women.

J Gend Specif Med. 2000 Jan-Feb;3(1):36-40

Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality.

To investigate the role of androgens in increasing bone density and improving low libido in postmenopausal women, we have studied the long-term effects of estradiol and testosterone implants on bone mineral density and sexuality in a prospective, 2 year, single-blind randomised trial. Thirty-four postmenopausal volunteers were randomised to treatment with either estradiol

implants 50 mg alone (E) or estradiol 50 mg plus testosterone 50 mg (E&T), administered 3-monthly for 2 years. Cyclical oral progestins were taken by those women with an intact uterus. Thirty-two women completed the study. BMD (DEXA) of total body, lumbar vertebrae (L1-L4) and hip area increased significantly in both treatment groups. BMD increased more rapidly in the testosterone treated group at all sites. A substantially greater increase in BMD occurred in the E&T group for total body ($P < 0.008$), vertebral L1-L4 ($P < 0.001$) and trochanteric ($P < 0.005$) measurements. All sexual parameters (Sabbatsberg sexual self-rating scale) improved significantly in both groups. Addition of testosterone resulted in a significantly greater improvement compared to E for sexual activity ($P < 0.03$), satisfaction ($P < 0.03$), pleasure ($P < 0.01$), orgasm ($P < 0.035$) and relevancy ($P < 0.05$). Total cholesterol and LDL-cholesterol fell in both groups as did total body fat. Total body fat-free mass (DEXA, anthropometry, impedance) increased in the E&T group only. We concluded that in postmenopausal women, treatment with combined estradiol and testosterone implants was more effective in increasing bone mineral density in the hip and lumbar spine than estradiol implants alone. Significantly greater improvement in sexuality was observed with combined therapy, verifying the therapeutic value of testosterone implants for diminished libido in postmenopausal women. The favourable estrogenic effects on lipids were preserved in women treated with T, in association with beneficial changes in body composition.

Maturitas . 1995 Apr;21(3):227-36

Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women--a clinical research center study.

Abdominal fat distribution is influenced by androgen levels in both men and women. The purpose of this study was to assess the effects on fat distribution of administering nandrolone decanoate (ND; an anabolic steroid with weak androgenic activity) or spironolactone (SP; an antiandrogen) in obese postmenopausal women. The design was a randomized, placebo-controlled, 9-month trial with simultaneous calorie restriction for weight loss. Women in all three groups lost comparable amounts of weight, but the ND-treated women gained lean mass relative to the other two groups ($P < 0.0005$) and lost more body fat than women in the SP group ($P < 0.01$). The resting metabolic rate also increased slightly in the ND group. ND treatment produced a gain in visceral fat, as determined by computed tomography scan, and a relatively greater loss of sc abdominal fat. SP-treated women lost significantly less sc fat than the other two groups. Serum cholesterol decreased in the placebo group, but increased slightly in the other two groups (significant for SP vs. placebo, $P < 0.05$). High density lipoprotein cholesterol decreased significantly in the ND-treated women. There were no significant changes in fasting glucose or insulin sensitivity. We conclude that administration of exogenous androgens modulates body composition in obese postmenopausal women and independently affects visceral and sc abdominal fat.

J Clin Endocrinol Metab . 1996 Jun;81(6):2198-203

Testosterone deficiency: a key factor in the increased cardiovascular risk to women following hysterectomy or with natural aging?

The ovaries are a critical source not only of estrogen but also of testosterone. On removal of the uterus, even in instances where ovaries have been spared, their function can be compromised. Women who have had a simple hysterectomy (ovaries remaining intact), even if treated postsurgically with supplementary estrogen, have three times the risk of cardiovascular disease compared with women who have not had a hysterectomy. In men, testosterone has been demonstrated to have beneficial fibrinolytic effects and beneficial effects on blood vessel endothelium, in blood sugar and insulin metabolism, and in maintaining coronary artery circulation. Studies on the potential cardiovascular protective effects of physiologic levels of testosterone in women are critically needed. Restoring a physiologic level of testosterone to women after hysterectomy not only can improve quality of life in terms of sexual libido, sexual pleasure, and sense of well-being but also can build bones--and may be a key to protecting cardiovascular health. Women developing testosterone deficiency as a consequence of natural aging/menopause may similarly benefit from physiologic testosterone supplementation.

J Womens Health . 1998 Sep;7(7):825-9

Serum sex hormone levels after menopause and subsequent breast cancer.

BACKGROUND: High levels of androgens and estrogens have been reported to be associated with breast cancer. However, the multiplicity of factors that influence hormone levels and methodologic issues complicate the study of the relationship between steroid sex hormones and breast cancer. **PURPOSE:** Using an improved study design, we assessed prospectively the relationship between the principal steroid sex hormones in serum and the subsequent occurrence of invasive breast cancer in postmenopausal women. **METHODS:** Four thousand fifty-three healthy postmenopausal women aged 40-69 years, were enrolled from June 1987 through June 1992 in a prospective investigation of hormones and diet in the etiology of breast tumors (ORDET study) as part of a larger volunteer cohort of 10 788 premenopausal and postmenopausal women from Varese Province, northern Italy. At recruitment, blood samples were taken between 8:00 AM and 9:30 AM (after overnight fasting), and sera were preserved in -80 degree Celsius freezers. Women who had received hormone treatment in the 3 months prior to enrollment, who had bilateral ovariectomy, or who had a history of cancer or liver disease were not recruited. Twenty-five women in the final eligible cohort of postmenopausal women developed histologically confirmed, invasive breast cancer during the first 3.5 years of follow-up for the cohort (13 537 women-years). For each case subject, four control subjects were randomly chosen after matching for factors possibly affecting hormone preservation in serum. One case subject and eight control subjects were excluded because premenopausal hormonal patterns were found; thus, after also excluding the four control subjects matched to the ineligible case

subject, we included 24 case and 88 control subjects. In the spring of 1994, stored sera of case and control subjects were assayed in a blinded manner for dehydroepiandrosterone sulfate and estradiol (E2) by in-house radioimmunoassay and for total and free testosterone and sex hormone-binding globulin by commercially available nonextraction iodination kits. Mean differences in risk factors were tested by analysis of variance for paired data. Relative risks (RRs) were estimated by conditional logistic regression analysis. All P values resulted from two-sided tests. RESULTS: Age-adjusted mean values of total testosterone, free testosterone, and E2 were significantly higher in case subjects than in control subjects: total testosterone, 0.34 ng/mL versus 0.25 ng/mL ($P < .001$); free testosterone, 1.07 pg/ml versus 0.77 pg/mL ($P = .006$); and E2, 25 pg/mL versus 22 pg/mL ($P = .027$). Age-adjusted RRs for breast cancer in increasing tertiles were as follows: for total testosterone, 1.0, 4.8, and 7.0 (P for trend = .026); for free testosterone, 1.0, 1.8, and 5.7 (P for trend = .005); and for total E2, 1.0, 7.1, and 5.5 (P for trend = .128). CONCLUSIONS AND IMPLICATIONS: This prospective study provides further evidence in support of the already established association between elevated estrogen levels and breast cancer. Even more importantly, it provides new evidence that high serum testosterone levels precede breast cancer occurrence.

J Natl Cancer Inst . 1996 Mar 6;88(5):291-6

ABSTRACTS

Sonograms

Ionizing radiation and cancer risk: evidence from epidemiology.

Epidemiological studies provide the primary data on the carcinogenic effects of radiation in humans. Much of what is known has come from studies of the atomic bomb survivors, and to a lesser extent from patients receiving radiotherapy. These studies demonstrate that exposure to moderate to high doses of radiation increases the risk of cancer in most organs. For all solid cancers combined, cancers of the thyroid, breast and lung, and leukemia, risk estimates are fairly precise, and associations have been found at relatively low doses (<0.2 Gy). Associations between radiation and cancers of the salivary glands, stomach, colon, bladder, ovary, central nervous system and skin have also been reported, but the relationships are not as well quantified. Associations between radiation and cancers of the liver and esophagus, and to a lesser extent multiple myeloma and non-Hodgkin's lymphoma, have been reported in a few studies, but results are inconsistent. Chronic lymphocytic leukemia, Hodgkin's disease, and cancers of the pancreas, prostate, testis and cervix have rarely been linked to radiation exposure. A linear no-threshold model adequately describes the dose-response relationship for solid cancers, although at extremely high doses the risk appears to flatten out. Because few populations have been followed until the end of life, the temporal patterns of risk are not completely known. An increased risk, however, does continue for several decades. In contrast, radiation-related leukemias begin to occur shortly (2-3 years) after exposure and, at least in the A-bomb survivors, a linear-quadratic dose response seems to fit the data better than a pure linear model. Radiation does not act entirely in isolation. It can interact with other carcinogens, e.g. tobacco or chemotherapeutic agents, and with host factors such as age at exposure, gender or reproductive history. Interactions with medical interventions or with certain heritable mutations have also been suggested. While the studies of high-dose exposures are essential for understanding the overall biological consequences of radiation exposure, the public is more concerned about the long-term health effects from protracted exposures at low doses. Unfortunately, the inherent limitations of epidemiology make it extremely difficult to directly quantify health risks from these exposures. While most epidemiological data are compatible with linear extrapolations from exposures at high doses or high dose rates, they cannot entirely exclude other possibilities. As the field of epidemiology advances, understanding more about the health effects of prolonged and low-dose exposures will be the next challenge.

Radiat Res . 1998 Nov;150(5 Suppl):S30-41

The risk of breast cancer after irradiation of the thymus in infancy.

It is well established that exposure to ionizing radiation during or after puberty increases a woman's risk for breast cancer, but it is less clear whether exposure to ionizing radiation very early in life is also carcinogenic. We studied the incidence of breast cancer prospectively in a cohort of 1201 women who received x-ray treatment in infancy for an enlarged thymus gland and in their 2469 nonirradiated sisters. After an average of 36 years of follow-up, there were 22 breast cancers in the irradiated group and 12 among their sisters, yielding an adjusted rate ratio of 3.6 (95% confidence interval, 1.8 to 7.3). The estimated mean absorbed dose of radiation to the breast was 0.69 Gy. The first breast cancer was diagnosed 28 years after irradiation. The dose-response relation was linear (P less than 0.0001), with a relative risk of 3.48 for 1 Gy of radiation (95% confidence interval, 2.1 to 6.2) and an additive excess risk of 5.7 per 10(4) person-years per gray (95% confidence interval, 2.9 to 9.5). We conclude that exposure of the female breast to ionizing radiation in infancy increases the risk of breast cancer later in life.

N Engl J Med. 1989 Nov 9;321(19):1281-4

Breast cancer in women with scoliosis exposed to multiple diagnostic x rays.

Although exposure to ionizing radiation is a recognized risk factor for breast cancer, the potential hazard from low-dose, fractionated exposures during early breast development has not been thoroughly evaluated. Women with scoliosis represent a valuable population for studying this issue because they are exposed to multiple diagnostic x rays during childhood and adolescence, times when the breast may be highly sensitive to the carcinogenic effects of radiation. A study was conducted of 1,030 women with scoliosis who were seen at four Minneapolis area medical facilities between 1935 and 1965. The average age at diagnosis was 12.3 years; 60% of the women had idiopathic scoliosis. Individual x-ray films were counted and the number per patient ranged from 0 to 618 films (mean, 41.5). On average, the x-ray exposures were given over an 8.7-year period. Ninety percent of the women were located, of whom over 92% responded to a mail questionnaire or telephone interview. The average period of observation was 26 years. Overall, 11 cases of breast cancer were reported, compared with six expected (standardized incidence ratio = 1.82, 90% confidence interval = 1.0-3.0). Excess risk increased with time since exposure and was highest among those followed for more than 30 years (standardized incidence ratio = 2.4). Risk also increased with the number of x rays and with the estimated radiation dose to the breast (mean, 13 rad). These data suggest that frequent exposure to low-level diagnostic radiation during childhood or adolescence may increase the risk of breast cancer.

J Natl Cancer Inst. 1989 Sep 6;81(17):1307-12

Incidence and occurrence of total (first-ever and recurrent) stroke.

BACKGROUND AND PURPOSE: It has recently been hypothesized that the figure of approximately half a million strokes substantially underestimates the actual annual stroke burden for the United States. The majority of previously reported studies on the epidemiology of stroke used relatively small and homogeneous population-based stroke registries. This study was designed to estimate the occurrence, incidence, and characteristics of total (first-ever and recurrent) stroke by using a large administrative claims database representative of all 1995 US inpatient discharges. **METHODS:** We used the Nationwide Inpatient Sample of the Healthcare Cost and Utilization Project, release 4, which contains approximately 20% of all 1995 US inpatient discharges. Because the accuracy of International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) coding is suboptimal, we performed a literature review of ICD-9-CM 430 to 438 validation studies. The pooled results from the literature review were used to make appropriate adjustments in the analysis to correct for some of the inaccuracies of the diagnostic codes. **RESULTS:** There were 682 000 occurrences of stroke with hospitalization (95% CI 660 000 to 704 000) and an estimated 68 000 occurrences of stroke without hospitalization. The overall incidence rate for occurrence of total stroke (first-ever and recurrent) was 259 per 100,000 population (age- and sex-adjusted to 1995 US population). Incidence rates increased exponentially with age and were consistently higher for males than for females. **CONCLUSIONS:** We conservatively estimate that there were 750,000 first-ever or recurrent strokes in the United States during 1995. This new figure emphasizes the importance of preventive measures for a disease that has identifiable and modifiable risk factors and for the development of new and improved treatment strategies and infrastructures that can reduce the consequences of stroke.

Stroke. 1999 Dec;30(12):2523-8

Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group.

BACKGROUND: The combined thickness of the intima and media of the carotid artery is associated with the prevalence of cardiovascular disease. We studied the associations between the thickness of the carotid-artery intima and media and the incidence of new myocardial infarction or stroke in persons without clinical cardiovascular disease. **METHODS:** Noninvasive measurements of the intima and media of the common and internal carotid artery were made with high-resolution ultrasonography in 5,858 subjects 65 years of age or older. Cardiovascular events (new myocardial infarction or stroke) served as outcome variables in subjects without clinical cardiovascular disease (4,476 subjects) over a median follow-up period of 6.2 years. **RESULTS:** The incidence of cardiovascular events correlated with measurements of carotid-artery intima-media thickness. The relative risk of myocardial infarction or stroke increased with intima-media thickness ($P < 0.001$). The relative risk of myocardial infarction or stroke (adjusted for age and sex) for the quintile with the highest thickness as compared with the lowest quintile was 3.87 (95% confidence interval, 2.72 to 5.51). The association between cardiovascular events and intima-media thickness remained significant after adjustment for traditional risk factors, showing increasing risks for each quintile of combined intima-media thickness, from the second quintile (relative risk, 1.54; 95% confidence interval, 1.04 to 2.28), to the third (relative risk, 1.84; 95% confidence interval, 1.26 to 2.67), fourth (relative risk, 2.01; 95% confidence interval, 1.38 to 2.91), and fifth (relative risk, 3.15; 95% confidence interval, 2.19 to 4.52). The results of separate analyses of myocardial infarction and stroke paralleled those for the combined end point. **CONCLUSIONS:** Increases in the thickness of the intima and media of the carotid artery, as measured noninvasively by ultrasonography, are directly associated with an increased risk of myocardial infarction and stroke in older adults without a history of cardiovascular disease.

N Engl J Med. 1999 Jan 7;340(1):14-22

Carotid atherosclerosis and ischemic stroke in young patients.

BACKGROUND: Epidemiological studies indicate a high prevalence of carotid atherosclerosis in elderly patients with ischemic stroke. The aim of this study was to investigate the presence of early carotid atherosclerotic lesions in young subjects with ischemic stroke, in the absence of the common atherosclerotic risk factors. **METHODS:** We studied 98 young patients with first ischemic stroke (54 males and 44 females; mean age 41.2 years; range 32-50) and 96 healthy controls. All subjects underwent ultrasonographic scanning of the carotid arteries according to a standardized protocol. **RESULTS:** The carotid intima-media thickness was significantly increased in the patient group ($p < 0.001$) compared with controls. In addition, the prevalence of carotid atherosclerotic plaques was greater in the patients than in the controls ($p < 0.001$). In particular, we detected 18 non-occlusive carotid plaques and 16 thrombotic occlusions. In eight patients, the lesions were bilateral. The echographic pattern of the plaques was hard in eight cases, soft in five cases, and mixed in the remaining five cases. **CONCLUSIONS:** We detected an increased wall thickness of the carotid arteries and an increased prevalence of carotid atherosclerotic lesions and carotid thrombotic occlusions in young patients with ischemic stroke, with a relative low incidence of cardiovascular risk factors. This finding suggests that arterial intima-media thickness per se is an important determinant of vascular disease in young patients. The data also provide indirect support for the potential role of genetic factors in the genesis of atherosclerosis in young patients.

Int Angiol. 2002 Jun;21(2):117-22

Transcranial Doppler and risk of recurrence in patients with stroke and patent foramen ovale.

The importance of patent foramen ovale (PFO) in stroke of unknown cause remains disputed, as PFO may be present in up to 20% of normal people and in a high proportion of patients with non-vascular disorders. Recent evidence suggests that the amount of right-to-left shunt (RLS) may be the crucial factor for stroke occurrence and relapse. The aim of the study was to assess

predictors of recurrence in PFO-related stroke patients on amount of shunting. Patients less than 61 years old who had been admitted for a PFO-related stroke within the previous five years, were re-evaluated on a follow-up visit. The clinical syndrome, residual disability, vascular risk factors and number of relapses as the index event were assessed. RLS sizing was semi-quantitatively performed with saline-enhanced transcranial Doppler (TCD), by assuming a cut-off of more or less 10 bubbles recorded in the cerebral vessels as a criterion to discriminate large versus small shunt, respectively. Thereafter patients were prospectively followed-up for a median time of 23 months. Total follow-up was 61 months. Fifty-nine patients (M/F = 23/36, mean age 43 +/- 13) were studied. Overall there were 23 relapses in 13 patients. The amount of shunting was the only significant independent variable associated with relapse: at the end of the follow-up period the recurrence rate was 0.66 and 8.2% per patient per year in patients with small and large shunt, respectively. This difference was statistically significant ($\chi^2 = 10.39$, $P = 0.0012$; OR 17.05, 95% CI 2.10-755.22). In patients with PFO-related stroke, the amount of RLS as assessed with TCD is the only independent predictor of relapse. PFO sizing is mandatory in patients with PFO.

Eur J Neurol . 2003 Mar;10(2):129-35

The importance of Doppler studies in asymptomatic intracranial and extracranial arterial disease.

Knowledge of intracranial and carotid disease in the symptomatic and especially the asymptomatic high risk population may be useful for evaluating future treatment modalities. A group of 204 symptomatic patients and 105 asymptomatic elderly patients at high risk for stroke were tested by carotid duplex ultrasound and transcranial doppler for the presence of carotid and intracranial stenosis. Quantitative measurements of the stenosis were made directly from the hard copy of the carotid duplex and the transcranial doppler. Of the 204 symptomatic patients 168 (83%) had some degree of stenosis: 84 of the 204 (41%) in the intracranial circulation only, 59 (29%) in the internal carotid only, and 26 (13%) in both sets of vessels. Of the asymptomatic patients 85 (81%) had some degree of stenosis; 31 of 105 (30%) in the intracranial circulation only, 35 (33%) in the internal carotid only, and 19 (18%) in both sets of vessels. Statistical analysis did not reveal significant differences between the two groups. The large percentage of intracranial disease in the symptomatic as well as the asymptomatic population at high risk for stroke require further confirmation by good duplex studies of the intracranial circulation. This is important in order to create coherent treatment protocols.

Keio J Med . 2002 Dec;51(4):189-92

Using sonography to screen women with mammographically dense breasts.

OBJECTIVE: Mammographically dense breast tissue has been reported both as a cause of false-negative findings on mammography and as an indicator of increased breast cancer risk. We conducted this study to evaluate the role of breast sonography as a second-line screening test in women with mammographically dense breast tissue. **MATERIALS AND METHODS:** Between January 2000 and January 2002, 1,517 asymptomatic women with dense breasts and normal mammography and physical examination findings underwent physician-performed breast sonography as an adjunct screening test. Within the study group, 318 women had a first-degree family history or personal history of breast cancer. The high-risk subgroup comprised these women. The detection rate of breast cancer in this subgroup was compared with the detection rate in the remaining study population with baseline risk. **RESULTS:** Of 1,517 women examined, seven breast cancers were diagnosed (cancer-detection rate, 0.46%). Four carcinomas were detected in high-risk women and three in women with baseline risk. The cancer-detection rate in the subgroup of high-risk women was 1.3%, significantly higher ($p < 0.04$) than the cancer-detection rate of 0.25% in the baseline risk subgroup. All cancers were T1 (range, 4-12 mm; mean, 9.6 mm). Sentinel lymph nodes were negative for cancer in six of seven carcinomas. **CONCLUSION:** Screening breast sonography in the population of women with dense breast tissue is useful in detecting small breast cancers that are not detected on mammography or clinical breast examination. The use of sonography as an adjunct to screening mammography in women with increased risk of breast cancer and dense breasts may be especially beneficial.

AJR Am J Roentgenol . 2003 Jul;181(1):177-82

Transvaginal sonography as a screening method for the detection of early ovarian cancer.

From December 1987 to December 1993, 6,470 women underwent screening with transvaginal sonography (TVS) as part of the University of Kentucky Ovarian Cancer Screening Project. Two groups of women were eligible to participate in this investigation: (i) asymptomatic postmenopausal patients or patients >50 years of age, and (ii) asymptomatic women >30 years of age with a family history of ovarian cancer. Ovarian volume was calculated using the prolate ellipsoid formula (length x height x width x 0.523). An abnormal sonogram was defined by (1) an ovarian volume >10 cm³ in postmenopausal women or >20 cm³ in premenopausal women, and (2) a papillary or complex tissue projection into a cystic ovarian tumor. All women with an abnormal TVS had a repeat sonogram in 4-6 weeks. Patients with persistently abnormal scans had a serum CA-125 determination, tumor morphology indexing, and color Doppler sonography. Ninety patients (1.4%) with a persisting abnormality on TVS underwent exploratory laparotomy or laparoscopy for tumor removal. Thirty-seven patients had serous cystadenomas and six had primary ovarian cancers. Five patients had Stage IA ovarian cancer and one patient had Stage IIIB disease. Only one of the ovarian cancer patients had a palpable abnormality on pelvic examination, and none had an elevated (>35 u/ml) serum CA-125. All these patients are presently alive and well 1-5 years after conventional therapy. There was one false negative in this study, a 38-year-old white female who was noted to have a small ovarian cancer at the time of laparoscopic prophylactic oophorectomy 11 months after a normal scan. Over 17,000 screening years have been accrued and there have been no deaths from primary ovarian cancer.

in the screened population. A cost analysis of TVS screening is presented.

Gynecol Oncol. 1997 Jun;65(3):408-14

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